<Division of Environmental Chemistry> Molecular Microbial Science

Division of Environmental Chemistry
- Molecular Microbial Science -

http://www.kuicr.kyoto-u.ac.jp/labos/bm2/lab_J.html

Prof
ESAKI, Nobuyoshi
(D Agr)

Assoc Prof
KURIHARA, Tatsuo
(D Eng)

Assist Prof
MIHARA, Hisaaki
(D Agr)

Res Associate
KAZUOKA, Takayuki
(D Eng)

Guest Res Assoc
KWAK, Mi-Sun

Technicians(pt)

TANAKA, Yumi
UTSUNOMIYA, Machiko
HAYASHI, Misa
KITAYAMA, Kaori

Students

IGARASHI, Motoki (D3)
KURATA, Atsushi (D3)
MURAMATSU, Hisashi (D3)
ABE, Katsumasa (D1)
KAWAMOTO, Jun (D1)
MIYAKE, Ryoma (D1)
EBATA, Ichiro (M2)
ITO, Kosuke (M2)
KUROKAWA, Suguru (M2)
MURAI, Ken (M2)
NAGAYASU, Makiko (M2)
OMORI, Taketo (M2)
UEMURA, Tadashi (M2)
HATA, Chikako (M1)
INOMOTO, Yasushi (M1)
JITSUMORI, Keiji (M1)
MURAKAMI, Yoshiko (M1)
OSAKI, Motoharu (M1)
TAGO, Tsukasa (M1)
TANI, Yasushi (RS)
ZHANG, Wanjiao (RS)

Scope of Research

Structure and function of biocatalysts, in particular, pyridoxal enzymes and enzymes acting on xenobiotic compounds, are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of sulfur, selenium, and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, molecular structures and functions of psychrophilic enzymes and their application are under investigation.

Research Activities (Year 2004)

Presentations

Properties of an enzyme catalyzing the asymmetric reduction of 2-chloroacrylic acid, Kurata A, Kurihara T, Kamachi H, Esaki N, 2004 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 29 March.

Functional analysis of Suf proteins, Ano K, Kazuoka T, Mihara H, Kurihara T, Esaki N, 2004 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 29 March.

Identification of phosphorylated proteins by proteome analysis of a psychrotrophic bacterium, *Schewanella* sp. Ac10, Kawamoto J, Kurihara T, Kato T, Kitagawa M, Asada K, Kato I, Esaki N, 2004 Annual Meeting, Jpn. Biochem. Soc., 14 October.

Enzymatic synthesis of L-pipecolic acid by Δ^1-piperideine-2-carboxylate reductase from *Pseudomonas putida,* Muramatsu H, Mihara H, Yasuda M, Ueda M, Kurihara T, Esaki N, 2004 Annual Meeting, Jpn. Biochem. Soc., 14 October.

Grants

Esaki N, Construction and functional analysis of composite biocatalysts, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2001 - 31 March 2004.

Esaki N, Elucidation of the mechanisms of activation of an essential trace element, selenium, and its co-translational incorporation into polypeptide chains, Grant-in-Aid for Scientific Research (B), 1 April 2003 - 31 March 2005.

Esaki N, Isolation of novel cold-adapted microorganisms and exploitation of useful gene resources, Grant-in-Aid for Scientific Research (B), 1 April 2003 - 31 March 2005.

Yoshimura T, Physiological role of D-amino acids in

Phosphoproteome Analysis of a Psychrotrophic Bacterium, *Shewanella* sp. Ac10, to Elucidate its Cold-adaptation Mechanism

We tried to elucidate the cold-adaptation mechanism of a psychrotrophic bacterium, *Shewanella* sp. Ac10, isolated from Antarctic seawater by phosphoproteome analysis. Membrane proteins were extracted from the cells cultivated at 4°C and 18°C and separated by two-dimensional gel electrophoresis (2-DE). Phosphorylated proteins were detected by staining the 2-DE gels with Pro-Q Diamond and identified by peptide mass fingerprinting. Six and eight membrane proteins were found to be phosphorylated in the cells cultivated at 18°C and 4°C, respectively. One of the proteins phosphorylated at 4°C was identified as a homolog of TolC, which functions as an exit duct in *Escherichia coli.* Although the amount of the TolC homolog was not significantly affected by the cultivation temperature, it was more remarkably phosphorylated at 4°C than at 18°C. We also found two phosphorylated proteins specifically produced at 4°C. These phosphorylated proteins probably play a role in the adaptation of this bacterium to a cold environment.

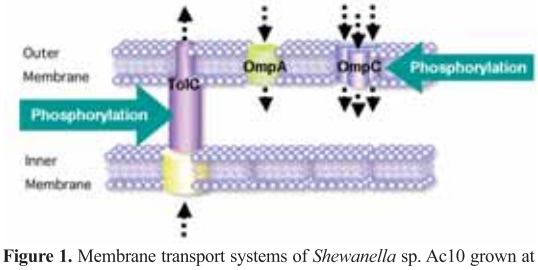

Figure 1. Membrane transport systems of *Shewanella* sp. Ac10 grown at 4°C.

Enzymatic Synthesis of *N*-methyl-L-phenylalanine by a Novel Enzyme, *N*-methyl-L-amino Acid Dehydrogenase

We found *N*-methyl-L-amino acid dehydrogenase activity in various bacterial strains, such as *Pseudomonas putida* and *Bacillus alvei,* and cloned the enzyme gene from *P. putida* ATCC12633 to *E. coli.* The enzyme purified to homogeneity from the recombinant *E. coli* catalyzed the NADPH-dependent formation of *N*-alkyl-L-amino acids from the corresponding α-keto acids (e.g., pyruvate, phenylpyruvate, and hydroxypyruvate) and alkylamines (e.g., methylamine, ethylamine, and propylamine). An enzymatic system for the synthesis of *N*-methyl-L-phenylalanine from phenylpyruvic acid and methylamine with *N*-methyl-L-amino acid dehydrogenase using NADPH and glucose dehydrogenase from *Bacillus subtilis* as a cofactor-recycling system is developed. Analysis of the product of the laboratory preparative scale process revealed *N*-methyl-L-phenylalanine in 98% yield and over 99% e.e. *N*-Methyl-L-phenylalanine can be used as chiral building blocks for the synthesis of several products with pharmacological activity.

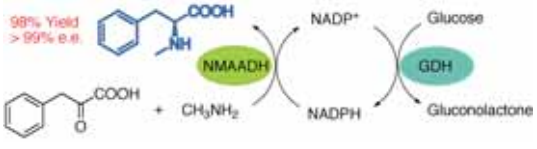

Figure 2. The enzyme-catalyzed production of *N*-methyl-L-amino acid in combination with cofactor-recycling catalyzed by glucose dehydrogenase.

eukaryote, Grant-in-Aid for Scientific Research (C), 1 April 2002 - 31 March 2004.

Kurihara T, Development of a low-temperature protein-production system regulating the formation of inclusion body, Grant-in-Aid for Exploratory Research, 1 April 2004 - 31 March 2006.

Kurihara T, Bioconversion of fluorinated organic compounds: catalytic mechanisms of elimination and incorporation of fluorine and their application, Grant-in-Aid for Young Scientists (A), 1 April 2002 - 31 March 2005.

Mihara H, Mechanisms of incorporation of sulfur and selenium into the anticodon wobble bases of tRNAs, Grant-in-Aid for Young Scientists (B), 1 April 2003 - 31 March 2006.

Kurihara T, Production of useful compounds and bioremediation of environments by cryobiotechnology using cold-adapted microorganisms, (NEDO), 1 April 2001 - 31 March 2004.

Kurihara T, In vivo and in vitro analysis of selenium metabolism - a multidisciplinary approach, Cooperative Research under the Japan-U.S. Cooperative Science Program (JSPS), 1 April 2001 - 31 March 2004.

Award

KURIHARA T, The Japan Bioscience, Biotechnology and Agrochemistry Society Award for the Encouragement of Young Scientists, Bioconversion of Organohalogen Compounds with Microbial Enzymes: Mechanistic Analysis of the Enzyme Reactions and Their Application, Jpn. Soc. Biosci. Biotech. Agrochem., 28 March 2004.